THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. INC.

110 East 59th Street
New York, N.Y. 10022

Application For Research Grant

Date: May 30, 1975

1. Name of Investigator(s): include Title and Degrees)

Albert Castro, Ph.D., Associate Professor of Pathology and Medicine
Director, Hormone Research Lab
University of Miami School of Medicine

Herbert Malkus, Ph.D., Assistant Professor of Pathology
Director, Clinical Chemistry
University of Miami School of Medicine
Jackson Memorial Hospital

2. Institution & Address:

University of Miami
School of Medicine
Department of Pathology
P.O. Box 520875, Biscayne Annex
Miami, Florida 33152

3. Short Title of Project: RADIOIMMUNOASSAY OF POLYAMINES MARKERS IN CANCER

4. Proposed Starting Date: January 1, 1976

5. Anticipated Duration of this Specific Study: 3 years

6. Brief Description of Objectives or Specific Aims:

The major objective of this proposal is to develop means of quantifying polyamines in special spermidine, spermine and putrecine as a marker in different types of malignant growth in animals and humans. In view of the low levels of the substance to be measured, and in view of our experience in detecting hormones, peptides and drugs, we believe that radioimmunoassay is the procedure of choice. The research proposal covers the development of specific antiserum against spermidine, spermine and putrecine. This methodology will be applied to measuring

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the levels of aliphatic polyamines, specific to patients with cancer of the lung, mammary and genital-urinary tract.

7. Give a Brief Statement of your Working Hypothesis: Appended

8. Details of Experimental Design and Procedures: (Attach Separate Pages) Appended

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

Appended

10. Additional Requirements:

Freezer to store biological material

Refrigerated centrifuge

Animals to produce antibodies. We will require rabbits and goats.

11. Biographical sketches of all principal and professional personnel (append)

Appended

12. List of publications: (Five most recent as pertinent)(append)

Appended

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13. Budget: (1st year)

		% time	Amount
A. Salaries (Personnel by names)			
Professional			
Albert Castro, Ph.D., Principal Investigator		20%	REDACTED
Herbert Malkus, Ph.D., CoPrincipal Invest.		30%	
Technical			
Laboratory Technician		100%	8,164
Retirement 8.95%			2,247
Fringe benefits - 5% for faculty	847		
7% for Staff	571		1,418
	Sub-Total		28,771
B. Consumable Supplies (list by categories			
Animals: 50 rabbits at $10 ea.			500
maintenance $0.30/day/rabbit, ave. 30 days			450
5 goats at $70 ea.			350
maintenance $1.50/day/goat, ave. 90 days			675
Chemicals and glassware			1,000
Radioactive isotopes			1,500
	Sub-Total		4,475
C. Other Expenses (itemize)			
Travel to Scientific Meetings, 1 trip			500
	Sub-Total		500
D. Permanent Equipment (itemize)			
PJR-2 refrigerated centrifuge with attachments			4,500
Freezer			500
	Sub-Total		5,000
E. Overhead (15% of A + B + C)			5,812
	Total		44,558

Estimated Future Requirements:

	Salaries	Consu.Suppl.	Other Exps.	Permanent Exps.	Ovhd.	Total
Year 2	31,073	4,922	500	0	5,474	41,969
Year 3	33,559	5,414	500	0	5,921	45,394

It is understood that the application and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [signature]
Director of Project 305/547/6508

Signature
Business Officer of the Institution
305/371/5572-Telephone

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

All current grants - $30,000 CTR - Radioimmunoassay of Nicotine

Pending -0-

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7. Working Hypothesis

Aliphatic polyamines such as spermidine, spermine and putrescine are widely distributed in nature. Their specific and vital physiological function as growth factors for certain microorganisms is emerging. The strong affinity that polyamines have for nucleic acid is attributed to neutralization of the phosphate group on the polynucleotides. Polyamines are naturally available intracellular stabilizers of biomembranes in many in vitro systems through formation of polyamine-phospholipid complexes.

Recently the spermine levels in urine in malignancy and pregnancy have been determined. The assays used in most of these studies were relatively insensitive and nonspecific. While their results are important, they should be re-evaluated with new, more precise methodologies to confirm their findings before the ultimate significance of polyamines as regulators of growth in neoplastic tissue is assigned. We propose for the determination of polyamines in biological materials on the basis of our previous experience the production of specific aliphatic polyamines antibodies and their use in the development of highly specific and sensitive radioimmunoassay.

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Background

Polyamines and cancer growth. Spermine and spermidine are present in all nucleated animal cells studied to date (1, 2, 3). In some rat tissues the spermidine-spermine ratio declines with the age of the animal (1, 4). The steady state concentration of putrescine in most mammalian tissue is almost always much lower than those of spermidine and/or spermine (3).

Even though the studies of the polyamines and their biological function increased during the last decade, not too much systematic attention has been given to polyamines and their biosynthetic enzymes in malignant cells. Significantly, Williams-Ashman et al (5) reported that in the most rapidly growing cell lines of the Morris hepatoma the concentration of putrescine were 5-10 times higher than those observed in the control liver. An increase in spermidine concentration with increased tumor growth rates were found to occur. The activity of ornithine decarboxylase was also noted to be significantly increased in all of the tumor studies and that continued synthesis of aliphatic polyamines in malignant cells occurred despite very rapid protein biosynthesis for other functions.

Spermine and spermidine have been shown to have potent inhibitory activity against neoplastic cells in vitro in the presence of calf serum containing spermine oxidase activity (6). 1003540540

Israel et al (7) found that some analogues of spermine and spermidine have shown significant in vitro anti-tumor activity against transplantable mouse tumors and also against human epidermoid carcinoma cells in culture systems containing calf serum.

Thirty-two analogues of spermine and spermidine were tested for significant anti-tumor activity. One analogue of spermine, a hydrochloride salt containing three methylenes between two secondary amino

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groups in the molecule, and three spermidine analogues containing on one side an aliphatic chain 3,5, or 9 methylenes long, were found to be active. Later the same authors proved that the toxic activity of these analogues is again ascribed to their oxidation products, especially dialdehyde. Quash et al reported that in animal tissues beta-amino-propionaldehyde can arise directly from the oxidation of spermidine or diaminopropane. They found that the serum level of the bound aldehyde was markedly elevated in patients with malignant metastasis. In contract the serum level of bound aldehyde in patients with nonmetastastic malignancy was significantly lower than compared with patients with either non-malignant or malignant diseases who had responded to treatment (8,9). It is of particular importance that β-aminopropianaldehyde can be found in animal tissue from the enzymatic oxidation of spermine and that the product is a constituent of normal human serum. On the basis of these findings Quash proposed that the lowering of β-amino-propianaldehyde (BAPA) levels could be caused by partial block in the reaction leading from spermidine to BAPA. The decreased substrate levels of BAPA thus could lead to a corresponding drop in the product malandialdehyde. Hence, more rapid growth takes place. If the reaction leading from BAPA to maloandialdehyde is now itself inhibited, then the small amount of BAPA which has been fed through this pathway can no longer be oxidized. Therefore, an increase in BAPA occurs.

In spite of the above observations Tabor and Tabor (10) make it clear that it is still not possible at this time to categorically state that the apparent relationship between polyamines and nucleic acids is actual. Polyamines do have an essential role in vivo but it will be necessary to clarify which of the many observations are really related to the physiological functions of the polyamines.

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Polyamines and their Presence in blood and urine. The presence of spermine in the serum in malignancy has been demonstrated (10). Spermine was found in the formed elements of normal human cells, principally in leucocytes, but none free in the plasma (11). Raina (12) found that spermine and spermidine were present in the whole blood but not in the plasma under ordinary circumstances. Methods for determinations of these polyamines have been superseded by our advancing technology, and deserve re-evaluation. Shiman (13) found that spermine occurred at high concentrations in red blood cells in vertebrates other than mammals but that concentrations were very low in iron-nucleated mammalian red blood cell lines.

Kosaki et al (14) isolated from malignant tissue a phospholipid called "malignolpin", which has been reported to contain spermine and to also be present in the sera of some cancer patients. Recently Quash (15, 16, 17), using anti-spermine antibodies, reported that serum from patients with cancer and chronic infections precipitate directly with antispermine antiserum, while those from patients with other pathological conditions give negative reactions. This antigenicity in cancer patients appears to be directly reacting with anti-spermine antibodies and migrates in the region of the -lipoproteins and contains spermine. Its characteristics suggest a similarity to "malignolpin". Quash postulated that when a new methylation pattern is imposed any time on a cell, as may occur with certain viral infections or certain alkylating carcinogens, the cells react by overproducing a lipid component which is very acid for spermine, and which as a result has the lipospermine complex in them found in the serum of cancer patients. The Quash hypothesis is sophisticated and disciplined and deserves further effort to confirm and enlarge. Quash (18) also found in the study of the effect of antipolyamine antibodies on mammalian cells and tissue cultures, that these antibodies in the presence of a complement

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exerted a cytolytic effect on cells, and the morphological changes were associated with an increase of lysosomes. A mechanism to explain the increased lysosome content of cells having antibodies fixed to their surface is not evident. But according to Quash it may be that these lysosomes are involved in excision of blocked sites caused to make them susceptible to repair.

Spermine and spermidine or their acetyl derivatives have also been studied in urine. Nakajima et al (19) could not detect spermine and spermidine in the urine of normal children, but did consistently detect monoacetyl derivatives of spermidine. The presence of spermine, spermidine and putrescine in hydrolyzed urine of cancer patients was demonstrated by Russel et al (20) who found that patients with histologically diagnosed tumors and leukemias had several-fold greater values of polyamines in urine compared to the normals. Surgical removal of all or a portion of the tumor always leads to a decrease of polyamines in urine. Despite the encouraging studies to date, it has not been established that elevated urinary polyamines are associated with all kinds of human cancer.

Finally, because of problems of urine collection, renal clearance, renal amine oxidases and bacterial production of the polyamines, our thrust must be to individual plasma polyamine ratios against the background of total polyamines in a given sample.

Specific Aims

On the basis of previous findings we propose:

(a) A radioimmunoassay method be used to study the level of the polyamines, spermine, spermidine and putrescine in whole blood, plasma, and urine during normal growth in humans.

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(b) Study of the polyamine levels in cancer patients with different types of tumors during the development of the tumor and its treatment.

METHODS OF PROCEDURE

Two basic motivations will be involved in these investigations. First, elicitation of the minimum number of tests necessary to achieve an appropriate cancer detection and therapeutic monitoring program. Second, accumulation of information which will shed light on the three poorly defined pathways; spermine metabolism to the monoaldehyde; the glutathione conjugation; the acetylation pathways and their relationship to each other.

Production of Antibodies.

1) The carbodiimide procedure to produce antibodies to small molecules is one that has been used extensively (21). We have produced antibodies to many different small peptides and steroid molecules (22,23,24,25,26) by different conjugated antigens.

Aliphatic polyamines (spermidine, spermine, putrescine) will be conjugated to a macromolecular carrier (BSA or HSA) by a carbodiimide water soluble method using 1-ethyl-3-(-3-) dimethylaminopropyl)-carbodiimide Hcl or 1-cyclohexyl-3-(-2-mopholinyl-(4)-ethyl carbodiimide (27, 28).

The conditions of the reaction are as follows: The carrier (BSA or HSA), an excess of hapten, and reagents will be stirred together in aqueous solution for 30 minutes. The reaction is followed by dialysis and product isolation.

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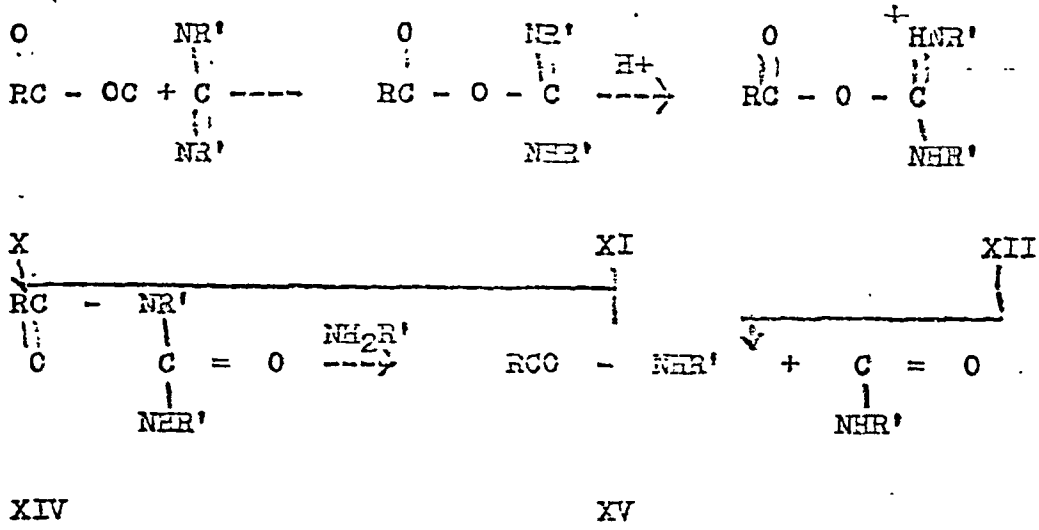
Reaction Mechanism



Reaction of carbodiimides with carboxylic acids

2) Conjugates obtained in this form will be injected into New Zealand white rabbits in 50% complete adjuvant via a) toe pad, b) intraperitoneal, 3) intramuscular.

Six weeks after the first injection, serum will be harvested, and by using labeled polyamines experiments on titer, specificity and affinity will be conducted.

Radioimmunoassay (Saturation analyses)

Radioimmunoassay is an extremely sensitive analytical method for detecting extremely low amounts of hormones, peptides, steroids, drugs and nucleotides. In practice this term has been applied to at least three methods whereby the use of a binding agent specific for the compound being determined is required, but these methods differ in the manner in which labeled compound is added and in the form of the calculations. In the other methods, or non-specific reactor or protein binding (CPB) one uses a non-specific protein with non-specific binding capacity to different molecules.

The two methods which will be used for development of radioimmunoassay for spermine and other aliphatic polyamines will be as follows:

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1) Add a known excess of binding agent to the solution containing the compound to be determined. The compound will take up some of the binding sites, but will leave the remainder free.

Add known amount of a highly specific labeled form of the same compound more than sufficient to saturate the remaining sites on the binding agent. Provided that the binding, once it occurs, is irreversible, then some of the radioactivity molecule will be bound and some will remain free. The ratio of free to bound molecules ($R \frac{F}{B}$) will be related to the number of sites on the binding agent which are taken up by the inactive molecules, that is, to the number of inactive molecules present. By calibrating the binding agent with known amounts of material the procedure can be made quantitative.

2) The other procedure involves mixing the compound to be determined and its labeled tracer before the binding agent is added. This means that instead of the inactive molecules being bound and the active molecule distributed between the remaining sites and the free state, the active and inactive molecules compete for the binding sites in equal basis. This does not require irreversible reaction between the molecule and the binding agent, and is in many ways similar to the substoichiometric dilution analysis.

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WORK ACCOMPLISHED BY US AND/OR OTHERS

A. We have in the last two years developed the majority of methods for radioimmunoassay in the literature, especially those related to hormones, peptides, steroids and drugs, and their application to clinical research studies. Our basic expertise lies in hooking up small molecules (low molecular weight) to many and varied macromolecular carriers by different chemical reaction techniques and producing specific high titer antibodies and developing highly sensitive specific radioimmunoassays for these compounds. (See publications in curriculum vitae.)

B. The detection of polyamines in body fluids has been of importance only recently, and because of the small quantities that are found, the techniques by which they have been measured are varied and non-specific.

1. The determination of polyamines in urine by gas-liquid chromatography/C.W. Gehrke, K.C. Kuo, R.W. Zumwalt, and T.P. Waalkes.
2. Gas chromatography-mass spectrometry (GC-MS) of diand polyamines in human urine; identification of monoacetylspermidine as a major metabolic product of spermidine in a patient with acute myelocytic leukemia/T. Walle.
3. Elevated polyamine levels in serum and urine of cancer patients: Detection by a rapid automatec technique utilizing an amino acid analyzer/L.J. Marton, J.G. Vaughn, I.A. Hawk, C.C. Levy, and D.H. Russell.
4. Clinical application of new methods of polyamine analysis/ M.D. Denton, H.S. Glazer, T. Walle, D.C. Zellner, and F. G. Smith.

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Although polyamines have been studied for many years, until recently they have been of importance in fields related to growth. (Also see bibliography of grant application.)

1. Janne, J., Raina, A., Siimes, M.: Spermidine and spermine in rat tissues at different ages. Acta. Physiol. Scand. 62: 352-358, 1964.

2. Caldarera, C.M., Moruzzi, M.S., Rossoni, C., Barbiroli, B.: Polyamines and nucleic acids metabolism during development of chick embryo brain. J. Neurochem. 16: 309-316, 1969.

3. Bachrach, V.: Metabolism and function of spermine and related polyamines, Ann. Rev. Microbiol. 24: 109-134, 1970.

4. Russell, D.H., Medina, V.J., Snyder, S.H.: The dynamics of synthesis and degradation of polyamines in normal and regenerating rat liver and brain, J. Biol. Chem. 245: 6732-6738, 1970.

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REFERENCES

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2. Russell, D.H., Snyder, S.H.: Amine synthesis in regenerating rat liver: extremely rapid turnover of ornithine decarboxylase, Molec. Pharmacol. 5: 253-262, 1969.

3. Pegg, A.E.: The effects of diamines and polyamines on enzymic methylation of nucleic acids. Biochem. Biophys. Acta. 232: 630-641, 1972.

4. Masayoshi, O., Inoue, H., Suzuki, F., Takeda, Y.: Studies on ornithine decarboxylase from the liver of thioacetamide-treated rats. Purification and some properties. Biochem. Biophys. Acta. 284: 285-297, 1972.

5. Williams-Ashman, H.G., Janne, J., Coppoc, G.L., Goroch, M.E., Schenone, A.: New aspects of polyamine biosynthesis in enkaryotic organisms. Advan. Enzyme Regulation 10: 225-245, 1972.

6. Russell, D.H., Snyder, S.H.: Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo and various tumors. Proc. Natl. Acad. Sci. USA 1420-1427, 1968.

7. Israel, M., Rosenfield, J.S., Modest, E.J.: Analogs of spermine and spermidine. I. Synthesis of Polymethylene-polyamines by reduction of cyanoethylated alpha, omega-alkylenediamines. J. Med. Chem. 7: 710-716, 1964.

8. Williams-Ashman, H.G., Coppoc, G.L., Weber, G.: Imbalance in ornithine metabolism in hepatomas of different growth rates as expressed in formation of putrescine, spermidine, and spermine. Cancer Res. 32: 1924-1932, 1972.

9. Israel, M., Zoll, E.C., Muhammad, N., Modest, E.J.: Synthesis and anti-tumor evaluation of the presumed sytotoxic metabolites of spermine and N,N'-bis (3-amino-propyl) nonane-1, 9-diamine. J. Med. Chem. 16: 1-5, 1973.

10. Tabor, H., Tabor, C.W., Rosenthal, S.M.: The biochemistry of the polyamines: Spermidine and spermine. Ann. Rev. Biochem. 30: 579-604, 1961.

11. Rosenthal, S.M., Tabor, C.W.: The pharmacology of spermine and spermidine. Distribution and excretion. J. Phar. Exp. Ther. 116: 131-138, 1956.

12. Raina, A.: Spermine and spermidine in normal human blood. Scand. J. Clin. Lab. Invest. 14: 318-319, 1962.

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13. Shimizu, H., Kakimoto, Y., Sano, I.: The determination and distribution of polyamines in mammalian nervous system. J. Pharm. Expt. Therap. 143: 199-204, 1964.

14. Kosaki, T., Ikoda, T., Kotani, Y., Nakagawa, S., Saka, T.: A new phospholipid, malignolipin in human malignant tumors. Science 127: 1176-1177, 1958.

15. Quash, G., Makaraj, K.: Quantitative estimation of free and bound serum aldehyde. Clin. Chim. Acta 30: 13-16, 1970.

16. Quash, G.A., Wilson, H.B.: Polyamines in human serum. W.I. Med. J. 16:81-91, 1967.

17. Quash, G.A.: Immunochimie des polyamines. Compt. Rend. Acad. Sci. (Paris) 265D: 934-936, 1967.

18. Quash, G.A., Delain, E., Euppert, J.: Effect of anti-polyamine antibodies on mammalian cells in tissue culture. Expt. Cell. Res. 66: 426-432, 1971.

19. Nakajima, T., Zack, Y.F., Jr., Wolfgram, F.: N-mono-acetyl-spermidine as a normal constituent of urine. Biochim. Biophys. Acta. 184:651-652, 1969.

20. Russell, D.H., Levy, C.C., Schimpff, S.C., Hawk, I.A.: Urinary polyamines in cancer patients. Cancer Res. 31: 1555-1558, 1971.

21. Sela, M., Ungar-Waron, H., and Schechter, Y.: Uridine-specific antibodies obtained with synthetic antigens. Proc. Nat. Acad. Sci. USA. 52: 285-292, 1964.

22. Castro, A., Kutas, M., Bartos, D., Chung, A.: A direct progesterone radioimmunoassay. Experientia 29: 636-637, 1973.

23. Castro, A., Grettie, D., Bartos, D., and Bartos, F.: LSD radioimmunoassay. Research Communications in Pathology and Pharmacology 6: 879-886, 1973.

24. Castro, A., Shi, T., Chung, A.: A direct plasma testosterone radioimmunoassay. Experientia 29: 1447-1448, 1973.

25. Cheng, L.T., Kim, S.V., Chung, A., Castro, A.: Amphetamines: New Radioimmunoassay. FEBS Letters 36: 339-342, 1973.

26. Castro, A., Bartos, D., Jelen, B., Kutas, M.: A radioimmunoassay for deoxycorticosterone in human peripheral plasma using sephadex LH-20 chromatography. Steroids 22 (6):851-867, 1974.

27. Sheehan, J., Cruickshank, P., and Boshart, G.: A convenient synthesis of water-soluble carbodiimides. J. Org. Chem. 26: 2525-2528, 1961.

28. Sheehan, J., and Hlavka, J.: The use of water-soluble and basic carbodiimides in peptide synthesis. J. Org. Chem. 21: 439-441, 1956.

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CURRICULUM VITAE

ALBERTO CASTRO, Ph.D.

Address: REDACTED

Birthdate:

Marital Status: REDACTED

Education and Degrees:

1953-1958 B.S., University of Houston, Houston, Texas

1957-1958 M.T., Jefferson Davis Hospital, Baylor University Houston, Texas

1959-1962 Doctor, Biological Chemistry, University of El Salvador, El Salvador, Central America

Postgraduate Education and Training:

1966-1968 Postdoctoral Research Fellowship in Diabetes and Hypertension: University of Oregon Medical School, Portland, Oregon.

1968-1970 Senior Research Fellow in Diabetes and Metabolism: University of Oregon Medical School, Portland, Oregon.

Aug. 1967 Radioisotopes in Medicine, in vitro Test, a course taken at the Clinical Laboratory, University of Oregon Medical School under E. Hine, M.D.

1963 Gas and Thin-layer Chromatography, a summer course given by the Department of Chemistry, University of El Salvador, under the direction of Professor J. Kannan, Ph.D.

Oct. 1964 Medical and Dental Curriculum, a course given by Leo Medelsky, Ph.D. of the University of Chicago at the University of Mexico.

Mar. 1965 Curriculum Integration in Medical Sciences, a course given by Leo Medelsky, Ph.D., of the University of Chicago at the University of El Salvador.

1965 Techniques of Teaching Science at Graduate Level, a course given by Faculty of Humanities, University of El Salvador.

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1966 Cultural Anthropology Applied to Biological Science, a summer course given by Armando Piovesan, M.D. of the University of Sao Paulo, Brazil.

Special Honors:

1964 General Relator, III International Congress of Dental Education for the Association of Latin American Dental Schools (120 Universities) Mexico.

1966 Award and Membership in ATENEO, Academy of Science of El Salvador.

Fellow, American Institute of Chemists

Northwest Pediatric Research

1966-1970 NIH Senior Research Fellow

Listed in:

American Men of Science--Physical and Biological Sciences
American Men and Women of Science--Medical Sciences
Who's Who in the West
Who's Who in the Southeast
Who's Who in Florida
Personalities of the West and Midwest
Community Leaders and Noteworthy Americans

Academic Appointments:

1958-1960 Assistant Professor of Microbiology and Biochemistry, Dental and Chemistry Schools, University of El Salvador, Central America.

1960-1963 Associate Professor, Biochemistry and Microbiology, Medical and Dental Schools, University of El Salvador.

1965-1968 Professor and Head, Department of Basic Sciences, Dental School, University of El Salvador.

1965-1966 Coordinator-Director, University of El Salvador General Studies and Pre-professional Curriculum.

1964-1968 Director of Research in Basic Science, Dental School, University of El Salvador.

1965-1966 Co-director of Graduate Research, Faculty of Chemistry, University of El Salvador.

1969-1973 Assistant Professor of Pediatrics and Co-director of the Pediatrics Metabolic Laboratory, University of Oregon Medical School, Portland, Oregon.

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1973-pres Senior Scientist, Papanicolaou Cancer Research Institute, Miami, Florida.

1972-pres Associate Professor of Medicine and Associate Professor of Pathology, University of Miami School of Medicine.

1974-pres Director, Hormone Research Laboratory, University of Miami, School of Medicine.

Experience in Academic Administration:

1963-1965 Member of the Following Technical Committees, Medical and Dental Schools, Admissions, Research, Graduate Education, University of El Salvador.

1961-1966 Member, Board of Directors, Dental School, University of El Salvador.

1963-1965 Member, University Supreme Council of Education, University of El Salvador.

1965-1966 Director of Curriculum Commission, Dental School, University of El Salvador.

Coordinator of expenditures for Scientific equipment in new science buildings from loans provided by International Branch of Development and other international organizations (for the Central American Universities).

1964-1965 Chairman, Research and Scholarship Committee, University of El Salvador

For many years I was assistant to the President of the University of El Salvador and Dean of the Medical and Dental Schools, and Consultant to other faculty deans in matters related to administrative and academic problems. I developed new educational methods and curriculum integration approaches which are now in use in many Latin American universities.

Professional Offices and Activities:

1967-1969 Member, Board of Directors of ATENEO, Academy of Science of El Salvador.

1967-1969 Member, Special Commission for Biological Sciences, ATENEO.

1965 Secretary, I Central American and II Costa Rican Congress of Microbiology..

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Professional Societies:

American Association of Medical Technologists

Sociedad de Microbiologia Latino Americana

Colegio de Quimica Biologica de El Salvador

Tooth and Bone Research Society, London

Fellow, Royal Society of Tropical Medicine and Hygiene, London

American Association for the Advancement of Science (AAAS)

American Association for Microbiology

American Chemical Association

New York Academy of Sciences

Fellow, American Institute of Chemists

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Publications:

1. Castro, A., Alfaro, A: Predominent microorganisms in the root canal in dental patients. Revista Sociedad Dental III: 23-29, 1962.

2. Castro, A.: Tuberculosis and deciclocerine. Revista del Sanatorio Nacional de El Salvador I: 219-223, 1963.

3. Mendez, E., Castro, A.: New approach for the treatment of root canal therapy. Anales of 4th Dental Congress of Central America and Panama, 1964, FOCAP.

4. Mackay, P., Castro, A.: Development of the tooth germ of rats in tissue culture. Anales of 4th Dental Congress of Central America and Panama, 1964, FOCAP.

5. Saca, M., Castro, A.: Tooth germ studies of reptiles in tissue culture. Facultad Odontologia II: 15-20, 1965.

6. Ugarte, E., Castro, A.: Potencia de la lizocima ante las cepas bacterianas mas comunes en conductor radiculares. Congreso Centroamericano I: 7 December, 1965.

7. Castro, A., Mendez, J.E., Sevilla, R.F., Beguer, P.M.: Poder bactericida de los cementos de obturacion de los conductos radiculares ante diferentes cepas bacterianas. Congreso Centroamericano de Microbiologia I: 6 December, 1965. II:9.

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11. Castro, A., Buist, N.: Insulin/glucose ratio in diagnosis of islet cell adenomas in children. Clin. Res. XXI (2): 289, Feb., 1973.

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CURRICULUM VITAE

HERBERT MALKUS, PH. D., Assistant Professor, Department of Pathology, University of Miami School of Medicine, and Director of Clinical Chemistry, Jackson Memorial Hospital, Miami, Florida, 1973 to present.

Date of Birth: REDACTED

Marital Status: REDACTED

Academic Background:

B.S., Brooklyn College, Brooklyn, New York, 1964.

Ph.D., Iowa State University, Ames, Iowa,
Teaching Assistant, 1964-1966
Research Assistant, 1966-1968

Postdoctoral Fellow, University of Florida, Gainesville, Florida, September, 1968 to March, 1970.

Interim Assistant Professor, University of Florida, Gainesville, Florida, 1970-1971.

NIH Postdoctoral Fellow Trainee, Clinical Chemistry, Yale University, New Haven, Connecticut, 1971-1973.

Research Experience:

Kinetics and mechanisms of the disproportionation of Iodosobenzene.

Electron spin resonance studies of semidiones derived from aliphatic acyloins

Synthesis of aliphatic acyloins

Synthesis of ^{13}C substituted aliphatic acyloins.

Synthesis of tetracyclododecadienes and bird cage compounds

Electron spin resonance studies of cyclopropyl conjugation

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Research Experience: Synthesis of platinum and palladium organometallic complexes

Thyroid Function Tests. Radioimmunoassay of triiodothyronine

Automated methods of analyses

Honors and Societies:
American Chemical Society, 1962
Sigma Xi, 1964
Phi Lambda Upsilon, 1966; Vice-President, Theta Chapter, 1967-1968
Alpha Chi Sigma, 1970
American Association of Clinical Chemists, 1973

Publications:

1. Russell, G. A., and Malkus, H.: Cyclopropyl Semidiones. J. Amer. Chem. Soc. 89: 160, 1967.

2. Chalfont, G. R., and Malkus, H.: Cyclopropyl Nitroxide Radicals. Abstract 158th National Meeting of the American Chemical Society, New York, N.Y., 1969, orgn 124.

3. Battiste, M. A., Haywood-Farmer, J., Malkus, H., Seidl, P., and Winstein, S.: J. Amer. Chem. Soc. 92: 2144, 1970.

4. Malkus, H., Battiste, M. A., and White, R. M.: Radical Anions Derived from Alkyl-substituted 1,2,4,5-Tetrazines. Chem. Communications, 479, 1970.

5. Russell, G. A., Lawson, D. R., Malkus, H. L., and Whittle, P. R.: ^{13}C-Hyperfine Splitting in Semidiones. J. Chem. Physics 54: 2164, 1971.

6. Haywood-Farmer, J., Malkus, H., and Battiste, M. A.: Synthesis and Nuclear Magnetic Resonance Spectra of Some Bridged Polyhydromethanonaphthalenes. Bridge Proton Assignments. J. Amer. Chem. Soc. 94: 2209, 1972.

7. Malkus, H., and Donabedian, R. K.: Triiodothyronine Radioimmunoassay. Clin. chim. Acta 51: 191, 1974.

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Papers in Preparation:

1. Malkus, H., Seligson, D., and Seligson, H.: Estimated Free Thyroxine, an Improved Measurement of Free Thyroxine.

2. Jatlow, P., and Malkus, H.: An Improved G C Assay for Phenobarbital, Primidone and Diphenylhydantoin.

Areas of Interest:

1. Radioimmunoassay of hormones and steroids

2. Thin layer chromatography of alkaloid metabolites

3. Automation of enzyme analyses

4. Thyroid Function Tests

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